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(71)Applicant : INTERNATL REAGENTS CORP
(72)Inventor : HASHIGUCHI YOICHI
IKEDA MASAIKU
SUMIYAMA ISAO

(54) DETERMINATION OF COMPONENT IN LIPOPROTEIN FRACTION

(57)Abstract:
PURPOSE: To realize an automatic multichannel measurement by subjecting a deaggregated fraction of a specific lipoprotein to quantitative reaction and measuring the variation continuously.
CONSTITUTION: A fraction of a specific lipoprotein is aggregated and the component, contained in the fraction of lipoprotein not subjected to determination, is introduced to a reaction system not pertaining to quantitative reaction. The fraction of specific lipoprotein is then deaggregated by such extent as to allow the quantitative reaction or dissolved by such extent as to allow the measurement thereof thus stopping the quantitative reaction. The variation caused thereby is measured continuously in the automatic analyzer for clinical inspection thus realizing multichannel measurement. Since a known enzyme is employed as a reagent for determination, a large number of specimens can be processed simultaneously while shortening the reaction time. It is applicable to various analyzers and quite effective in the clinical inspection because micro quantity of sample is employed directly as a specimen in the simultaneous measurement of many terms and can be measured optically.

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* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] this invention relates to the determination technique, such as cholesterol in LDL fractionation aiming at use by the clinical test, especially about the technique of carrying out determination of the component contained in the specific fractionation of the lipoprotein in biological materials, such as a blood serum and plasma.
[0002]
[Description of the Prior Art] A lipid is combined with an apoprotein in the sanguis, and lipoprotein is formed and it is metabolized. Lipoprotein is usually classified into fractionations, such as chylomicrons (CM), very low density lipoprotein (VLDL), intermediate type lipoprotein (IDL), low-density lipoprotein (LDL), and high density lipoprotein (HDL), according to specific gravity. And various illnesses and factors affect the metabolism of these lipoprotein, and connect with an increase and a decrement in the blood of a lipoprotein fraction. Among these, it is known that LDL participates in a store of the cholesterol to a blood vessel wall, and an arteriosclerosis operation is shown, and generally the cholesterol in LDL fractionation is called bad cholesterol, and is measured for a prevention of arteriosclerosis, ischemic heart disease, etc., or a diagnosis.
[0003] As a measuring method of LDL fractionation, the precipitation method, the ultracentrifugal method, the electrophoresis method, etc. are known conventionally. Although the fractional precipitation of the precipitation method is carried out using precipitation reagents, such as a poly-anion, and LDL fractionation is measured, like a ultracentrifugal method and an electrophoresis method, operation is very complicated and there is a fault that it cannot use, by the automatic analyzer currently used widely in the field of the clinical test. Moreover, although there is also the technique (JP,58-45562,A) of adding the polyvinyl sulfate which has the cation of 1 **, an automatic analyzer cannot be used too.
[0004] Although various things are checked in the field of the clinical test as a component contained in a specific fractionation, it divides and cholesterol, triglyceride, phospholipid, etc. are known well. Among these, the technique using the enzyme reaction to which it can be used on conditions [**** / in recent years], and the specificity of a reaction is called high enzymatic process is chiefly used for the cholesterol determination in the case of coming out, for example, carrying out determination of the cholesterol. This can be divided roughly into the technique of measuring the absorbance in a visible region to cholesterol esterase (CE) and cholesterol oxidase (CO) combining a par oxidase (POD) and a chromogen, and the technique of measuring the absorbance in an ultraviolet region to CE and a cholesterol dehydrogenase (CHD) combining a coenzyme.
[0005]
[Problem(s) to be Solved by the Invention] By the measuring method of the conventional LDL fractionation, the application to an automatic analyzer cannot be performed as mentioned above. Since the preparative isolation process of the sample by centrifugal separation is required for a precipitation method, in addition to the ability not to perform automatical measurement by the multichannel, it also includes the risk of starting contamination accidentally at the time of preparative isolation. It is in the purpose of this invention offering the technique of carrying out determination of the component which improves an above-

mentioned trouble and is contained in the specific fractionation of the lipoprotein in a biological material by the automatic analyzer, and it divides and is in offering the useful determination technique of the cholesterol in LDL fractionation in a clinical test.
[0006]
[Means for Solving the Problem] When leading the component which this invention persons make condense a specific lipoprotein fraction as a result of inquiring zealously, is contained in lipoprotein fractions other than specific lipoprotein, and is contained in the concerned lipoprotein fraction of the specialization which should be carried out determination, and the same component to another system of reaction which does not participate in a determination reaction, since the lipoprotein of meantime specialization itself was condensing, it found out not acting on this another reaction. And as a result of repeating a research further, it turns out that solution flocculation is carried out at the grade which can carry out the determination reaction of the lipoprotein fraction of next specialization at least, the concerned component is given to a determination reaction, a specific lipoprotein fraction is melted in the grade which can measure a determination reaction by the case further, and a determination reaction can be stopped. And by measuring the concentration of the product produced by concentration change of change produced by this determination reaction, i.e., this component, and this reagent, and the reaction, for example, an extinction, and a photogene etc., the above-mentioned problem was solvable, and it turns out that the purpose of this invention can be attained, and came to complete this invention.
[0007] Namely, this invention is the technique of carrying out direct determination of the component contained in the specific lipoprotein fraction in a biological material. The component which is made to condense a specific lipoprotein fraction, is contained in lipoprotein fractions other than a specific lipoprotein fraction, and is contained in the concerned lipoprotein fraction of the specialization which should be carried out determination, and the same component are led to another system of reaction which does not participate in a determination reaction. Solution flocculation is carried out at the grade which can carry out the determination reaction of the specific lipoprotein fraction at least, the concerned component is given to a determination reaction, and the determination technique of the component contained in the lipoprotein fraction characterized by measuring change produced by the determination reaction is offered.
[0008] In order to make a specific lipoprotein fraction condense by this invention, it is suitable to use a flocculant and an antibody. A flocculant makes flocculation cause according to a chemical reaction, is an antibody [as opposed to the lipoprotein fraction of specialization / an antibody], and produces the immune agglutination here. Such a flocculant and an antibody can be combined simultaneously [, and], and can be used. [using independently by the fractionation made to condense] [adding others, after adding either previously] Various things besides the precipitation reagent used for the above-mentioned precipitation method can be used that what is necessary is just what can attain the purpose of this invention as such a flocculant. For example, the making LDL fractionation condense among the lipoprotein in the sanguis purpose, it is independent or a tungstophosphoric acid besides a polyethylene glycol (PEG), dextran sulfate, a heparin, etc. can be used combining cations, such as Mg^{++}, Mn^{++}, calcium^{++}, Li^{++}, and nickel^{++}.
[0009] Moreover, for the purpose to which the immunoagglutination of the LDL fractionation of lipoprotein is carried out, one sort of the polyclonal antibody or monoclonal antibody to LDL, appointment B, and beta-lipoprotein or two or more sorts can be used as an antibody to LDL fractionation that what is necessary is just what can attain the purpose of this invention as an antibody.
[0010] A well-known reagent can be used for the reagent used in order to detect and carry out determination of the component contained in a specific lipoprotein fraction by this invention in fields, such as a clinical test. For example, for the purpose which carries out determination of the cholesterol, the reagent using CE, CO, and POD in the above-mentioned enzymatic process is desirable.
[0011] Reaction time until it carries out the determination reaction of such a reagent with a

sample and it stops the concerned reaction changes with reagents to use. When CE, CO, and POD are used in order to be usually able to choose from for 1 - 60 minutes, for example, to carry out determination of the cholesterol although there were also what was measured at the terminal point, and a thing from which the result same as a determination value is obtained even if it stopped the reaction on the way, generally it can choose from for 2 - 30 minutes.
[0012] Although technique well-known for stopping the concerned determination reaction by this invention can be used, when based, for example on an enzymatic process, the inhibitor for making the enzyme check can also be used. It can choose from heavy metal, antiseptics, a protein modifier, a surfactant, etc. as such an inhibitor. A sodium azide, a guanidine hydrochloric acid, etc. can be raised with the determination of the cholesterol using CE, CO, and POD as an example.
[0013] After saturating a determination reaction instead of stopping a determination reaction, a component may be detected, or a component may be detected after fixed time from start of a reaction, and the amount of a component may be measured using the calibration curve created beforehand.
[0014] Various things can be raised with this invention as system of reaction for leading the component contained in a specific lipoprotein fraction, and the same component to another system of reaction which does not participate in a determination reaction. For example, when CE, CO, and POD are used in order to carry out determination of the above-mentioned cholesterol, although these required enzymes are included, if they make the thing except a part of reagent (chromogen) which participates in coloring react as a reagent, they will form colorless complex. Then, in order to perform the target determination reaction, even if it makes all of CE, COs, PODs, and chromogens react, colorless complex does not participate in this.
[0015] If HDAOS (formal name:N- (2-hydroxy-3-sulfo propyl) -3, 5-dimethoxy aniline) is made to act as CE, CO, POD, and a chromogen after making the specific fractionation of lipoprotein condense as this example The cholesterol ester and cholesterol which are this component contained in lipoprotein fractions other than a specific lipoprotein fraction generate a hydrogen peroxide by operation of CE and CO, and POD and HDAOS form colorless complex in response to this. Since the specific lipoprotein fraction itself is condensing at this time, it does not participate in this reaction. Next, the solution flocculation of the specific lipoprotein fraction is carried out at the grade on which CE and CO can act, and if the reagent for determination which consists of CE, CO, POD and HDAOS, and a 4-amino antipyrin is made to react, the cholesterol ester and cholesterol which are this component contained in a specific lipoprotein fraction will generate a hydrogen peroxide by operation of CE and CO, and will generate the coloring matter with which POD, a 4-amino antipyrin, and HDAOS have an absorption maximum on the wavelength near 580nm At this time, flocculation is melted in the grade which can measure the absorbance of this reaction, and the determination of the cholesterol contained in a specific lipoprotein fraction can be carried out by measuring an absorbance.
[0016] As other examples, the system of reaction which does not involve can also be used for a determination reaction at the reaction different from a determination reaction. For example, when using the above-mentioned enzyme reaction of CE, CO, and POD for a determination reaction, with the component of lipoprotein fractions other than a specific lipoprotein fraction, the system of reaction of CE, CHD, and NAD+ is made to act, and it leads to the system of reaction which makes NADH generate. Then, make CHD check, this reaction is made not to advance, a specific lipoprotein fraction is made to react with the reagent for determination which carries out solution flocculation and becomes the grade on which CE and CO can act from CE, CO, POD and HDAOS, and a 4-amino antipyrin, flocculation is melted in the grade which can measure the absorbance of this reaction as mentioned above, and the determination of the cholesterol contained in a specific lipoprotein fraction can be carried out by measuring an absorbance.
[0017] independent [in a surfactant, mineral etc.] as a resolvent, in order to make the grade which can carry out determination at least carry out the solution flocculation of the specific

lipoprotein fraction condensed by this invention — or two or more sorts can be used As an example of a resolvent, triton X, a ****** toll, a sodium chloride, potassium chloride, etc. can be mentioned. For example, after making LDL fractionation condense, in order to make the grade which can carry out determination at least carry out solution flocculation, a triton X-100 can be used. moreover, independent [in a protein modifier, a surfactant mineral, etc.] as a resolvent, in order to make it melt in the grade which can measure a determination reaction by the case -- or two or more sorts can be used Guanidine and its salt can be mentioned as the example.

[0018]

[Function and Effect of the Invention] By the technique of this invention, make a specific lipoprotein fraction condense and the component contained in the lipoprotein fraction aiming at determination is led to the system of reaction which does not participate in a determination reaction. Carry out the solution flocculation of the specific lipoprotein fraction, and the grade which can carry out a determination reaction at least is made it to carry out a determination reaction. Furthermore, it melts in the grade which can measure a determination reaction by the case, a determination reaction is stopped, and measurement by multichannel-izing using the automatic analyzer in the conventionally difficult clinical test is attained by performing continuously operation of measuring change produced by this determination reaction. Moreover, since a well-known enzyme can be used for the reagent for determination, reaction time can be shortened and multi-analyte processing is also possible.

[0019] Furthermore, in the conventional precipitation method, in order to make the supernatant liquid of centrifugal separation into an analyte, an excessive quantity of a sample is required, although the blood serum which is a sample is the need mostly, since a sample is made into a direct analyte by the technique of this invention, minute-amount-izing of the amount of analytes is possible, and it is suitable for the multi-item coincidence measurement. In addition, by the technique of this invention, since optical measurement is possible, the technique of this invention -- an application is possible to many analysis apparatus currently used widely in the field of the clinical test -- does a very useful effect so to a clinical test.

[0020]

[Example] Although an example is hereafter given in order to explain this invention to a detail more, this invention is not limited to these.

By the technique of example 1 this invention, the example which carries out determination of the cholesterol in LDL fractionation is shown. The following reagent was prepared first.

A reagent 1 : The anti-Homo-sapiens appointment B goat blood serum reagent 2 (R1) 20% : PEG4000, and 13 units / ml (R2) CE, 13 units / mlCO, and 1.0 units / ml POD and 0.2mg/ml N- (2 - hydroxy-3-sulfo propyl) -3, 5-dimethoxy aniline, the 0.05% triton X-100 reagent 3 (R3):1% triton X-100, a 0.11mg/ml 4-amino antipyrin chromogen reagent 4(R4):7M-guanidine hydrochloric acid, 1M-guanidine thiocyanate salt [0021] Using the above-mentioned reagent, the high price Homo-sapiens blood serum (S) of 158.4mg/dl was diluted, the sequence was made, it was made into the analyte, and the cholesterol in LDL fractionation measured with the pattern of drawing 1 by the Hitachi 7070 type automatic analyzer, and made each dilution sequence the graph. The result is shown in drawing 2 . According to the technique of this invention, this result shows that the cholesterol in LDL fractionation can improve [linearity] determination.

[0022] By the technique of example 2 this invention, determination of the cholesterol in LDL fractionation was carried out, and it compared with the determination result in the conventional technique. According to the technique of this invention, using the reagent of an example 1, the fresh Homo-sapiens blood serum (S) was similarly made into the analyte, and it measured with the pattern of drawing 1 by the Hitachi 7070 type automatic analyzer, and compared with the result which carried out determination of the cholesterol in LDL fractionation in these blood serums from the measured value of the standard solution by the assay [Clinical Chemistry (Clin.Chem.) 18, 499, and 1972] of the cholesterol in LDL fractionation. The result is shown in Table 1. [0023] This result showed that according to the technique of this invention the conventional free ******** method and conventional measured

value correlated well (correlation coefficient r=0.987), and the determination of the cholesterol in LDL fractionation could be carried out by the automatic analyzer.

[0024]

[Table 1]

血清（No.）	本発明の方法 (mg/dℓ)	公知の方法 (mg/dℓ)
1	108.3	121.9
2	163.5	181.4
3	78.3	85.5
4	91.5	96.8
5	145.1	160.1
6	131.5	128.4
7	49.8	59.0
8	136.7	150.2
9	150.3	162.4
10	129.5	137.0
11	92.0	92.9
12	129.4	124.4
13	119.5	122.4
14	153.1	164.2
15	195.0	207.2
16	116.1	123.5
17	116.7	134.3
18	186.4	203.3
19	183.5	190.9

[Translation done.]

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CLAIMS

[Claim(s)]
[Claim 1] It is the technique of carrying out direct determination of the component contained in the specific lipoprotein fraction in a biological material. The component which is made to condense a specific lipoprotein fraction, is contained in lipoprotein fractions other than a specific lipoprotein fraction, and is contained in the concerned lipoprotein fraction of the specialization which should be carried out determination, and the same component are led to another system of reaction which does not participate in a determination reaction. The determination technique of the component contained in the lipoprotein fraction which carries out solution flocculation at the grade which can carry out the determination reaction of the specific lipoprotein fraction at least, and is characterized by measuring change which gave the concerned component to the determination reaction and was produced by the determination reaction.
[Claim 2] Technique according to claim 1 a specific fractionation is low-density lipoprotein (LDL).
[Claim 3] Technique according to claim 1 or 2 the component which should be carried out determination is cholesterol.
[Claim 4] The technique according to claim 1 of performing, after saturating a determination reaction in measurement of change, or after stopping a determination reaction by the case after fixed time from start of a determination reaction.

[Translation done.]

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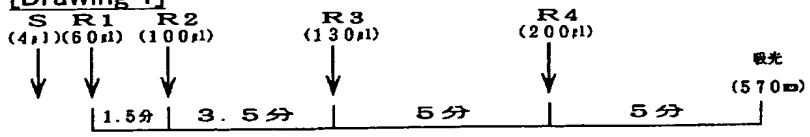
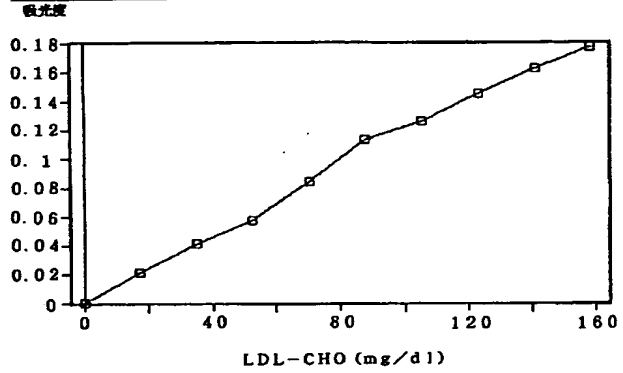
DRAWINGS

[Drawing 1]



[Drawing 2]



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Correction and a correction

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[Procedure correction 1]
[Document to be Amended] Specification
[Item(s) to be Amended] Claim
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[Claim(s)]
[Claim 1] It is the technique of carrying out direct determination of the component contained in the specific lipoprotein fraction in a biological material. The component which is made to condense a specific lipoprotein fraction, is contained in lipoprotein fractions other than a specific lipoprotein fraction, and is contained in the concerned lipoprotein fraction of the specialization which should be carried out determination, and the same component are led to another system of reaction which does not participate in a determination reaction. The determination technique of the component contained in the lipoprotein fraction which carries out solution flocculation at the grade which can carry out the determination reaction of the specific lipoprotein fraction at least, and is characterized by measuring change which gave the concerned component to the determination reaction and was produced by the determination reaction.
[Claim 2] The determination technique according to claim 1 that the aforementioned specific lipoprotein fraction is low-density lipoprotein (LDL).

[Claim 3] The assay of the cholesterol in LDL characterized by carrying out determination of the resultant which an enzyme reaction is made to act to the cholesterol in LDL, and is generated by the reaction from the sample containing low-density lipoprotein (LDL) after leading the cholesterol in lipoprotein other than LDL to another system of reaction which does not participate in a determination reaction alternatively.
[Claim 4] The determination technique according to claim 3 which consists of making the reagent which faces making an enzyme reaction act to cholesterol, and enables the reaction of the cholesterol in LDL exist.
[Claim 5] The determination technique according to claim 3 or 4 which is the technique on which an enzyme reaction is made to act to cholesterol by adding the reagent with which the method of leading the cholesterol in lipoprotein other than low-density lipoprotein (LDL) to another system of reaction which does not participate in a determination reaction makes LDL condense alternatively at least, and can generate a floc.
[Procedure correction 2]
[Document to be Amended] Specification
[Item(s) to be Amended] 0002
[Method of Amendment] Change
[Proposed Amendment]
[0002]
[Description of the Prior Art] A lipid is combined with an apoprotein in the sanguis, and lipoprotein is formed and it is metabolized. Lipoprotein is usually classified into fractionations, such as chylomicrons (CM), very low density lipoprotein (VLDL), low-density lipoprotein (LDL), and high density lipoprotein (HDL), according to specific gravity. And the factor of various illnesses affects the metabolism of these lipoprotein, and connects with an increase and a decrement in the blood of a lipoprotein fraction. Among these, it is known that LDL participates in a store of the cholesterol to a blood vessel wall, and an arteriosclerosis operation is shown, and generally the cholesterol in LDL fractionation is called bad cholesterol, and is measured for a prevention of arteriosclerosis, ischemic heart disease, etc., or a diagnosis.
[Procedure correction 3]
[Document to be Amended] Specification
[Item(s) to be Amended] 0005
[Method of Amendment] Change
[Proposed Amendment]
[0005]
[Problem(s) to be Solved by the Invention] By the measuring method of the conventional LDL fractionation, the application to an automatic analyzer cannot be performed as mentioned above. Since the preparative isolation process of the sample by centrifugal separation is required for settling, in addition to the ability not to perform automatical measurement by the multichannel, it also includes the risk of starting contamination accidentally at the time of preparative isolation. The purpose of this invention is to offer the technique of carrying out determination of the component which improves an above-mentioned trouble and does not need the preparative isolation process of a sample and which is contained in the specific fractionation of the lipoprotein in a biological material by the automatic analyzer, is divided and is to offer the useful determination technique of the cholesterol in LDL fractionation in a clinical test.
[Procedure correction 4]
[Document to be Amended] Specification
[Item(s) to be Amended] 0007
[Method of Amendment] Change
[Proposed Amendment]
[0007] Namely, this invention is the technique of carrying out direct determination of the component contained in the specific lipoprotein fraction in a biological material. The component which is made to condense a specific lipoprotein fraction, is contained in

lipoprotein fractions other than a specific lipoprotein fraction, and is contained in the concerned lipoprotein fraction of the specialization which should be carried out determination, and the same component are led to another system of reaction which does not participate in a determination reaction. Solution flocculation is carried out at the grade which can carry out the determination reaction of the specific lipoprotein fraction at least, the concerned component is given to a determination reaction, and the determination technique of the component contained in the lipoprotein fraction characterized by measuring change produced by the determination reaction is offered. Moreover, after this invention leads the cholesterol in lipoprotein other than LDL to another system of reaction which does not participate in a determination reaction alternatively from the sample containing low-density lipoprotein (LDL), it makes an enzyme reaction act to the cholesterol in LDL, and offers the assay of the cholesterol in LDL characterized by carrying out determination of the resultant generated by the reaction. It can face making an enzyme reaction act to cholesterol, and the reagent which enables the reaction of the cholesterol in LDL can be made to exist in here. Moreover, an enzyme reaction can be made to act to cholesterol by adding the reagent with which the method of leading the cholesterol in lipoprotein other than LDL to another system of reaction which does not participate in a determination reaction makes LDL condense alternatively at least, and can generate a floc.

[Translation done.]

文献2

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// Ｃ１２Ｑ	1/60		6807－4Ｂ		

審査請求　未請求　請求項の数４　ＯＬ　（全 5 頁）

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(71)出願人　000170565
国際試薬株式会社
兵庫県神戸市中央区浜辺通２丁目１番30号

(72)発明者　橋口　陽一
兵庫県神戸市西区室谷１丁目１－２　国際試薬株式会社研究開発センター内

(72)発明者　池田　昌郁
兵庫県神戸市西区室谷１丁目１－２　国際試薬株式会社研究開発センター内

(72)発明者　角山　功
兵庫県神戸市西区室谷１丁目１－２　国際試薬株式会社研究開発センター内

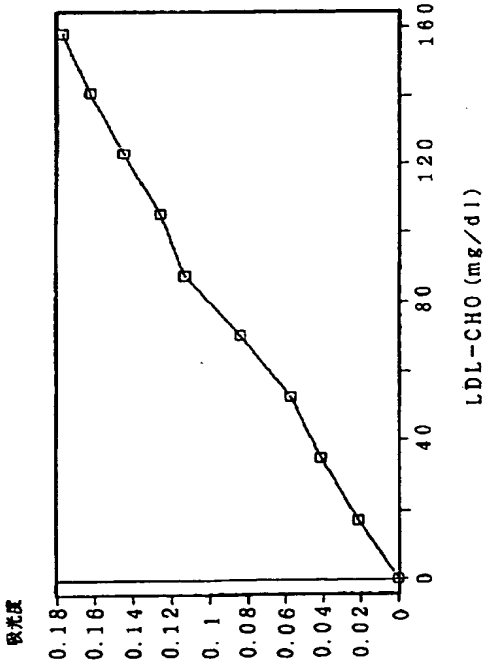
(74)代理人　弁理士　育山　葆　（外１名）

(54)【発明の名称】　リポ蛋白分画中の成分の定量方法

(57)【要約】

【構成】　生体試料中の特定のリポ蛋白分画に含まれる成分を、特定のリポ蛋白分画を凝集させ、　特定のリポ蛋白分画以外のリポ蛋白分画に含まれ当該定量すべき特定のリポ蛋白分画に含まれる成分と同じ成分を定量反応に関与しない別の反応系に導き、特定のリポ蛋白分画を少なくとも定量反応できる程度に解凝集して、当該成分を定量反応に付し、定量反応により生じた変化を測定することにより、直接定量する。

【効果】　従来困難であった臨床検査での自動分析装置を用いたマルチチャンネル化による測定が可能となる。また定量用の試薬には公知の酵素を用いることができるため、反応時間を短縮でき、多検体処理も可能である。試料を直接検体とするため検体量の微量化が可能で、多項目同時測定に適している。



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【特許請求の範囲】

【請求項１】　生体試料中の特定のリポ蛋白分画に含まれる成分を直接定量する方法であって、

①特定のリポ蛋白分画を凝集させ、

②特定のリポ蛋白分画以外のリポ蛋白分画に含まれ当該定量すべき特定のリポ蛋白分画に含まれる成分と同じ成分を定量反応に関与しない別の反応系に導き、

③特定のリポ蛋白分画を少なくとも定量反応できる程度に解凝集して、当該成分を定量反応に付し、

④定量反応により生じた変化を測定することを特徴とするリポ蛋白分画に含まれる成分の定量方法。

【請求項２】　特定分画が低比重リポ蛋白（ＬＤＬ）である請求項１に記載の方法。

【請求項３】　定量すべき成分がコレステロールである請求項１又は２に記載の方法。

【請求項４】　変化の測定を、定量反応が飽和した後、または定量反応の開始から一定時間後に、場合により定量反応を停止した後に行う請求項１記載の方法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、血清、血漿などの生体試料中のリポ蛋白の特定分画に含まれる成分を定量する方法に関し、とりわけ臨床検査での使用を目的とするＬＤＬ分画中のコレステロール等の定量方法に関する。

【０００２】

【従来の技術】血液中で脂質はアポ蛋白と結合し、リポ蛋白を形成して代謝される。リポ蛋白は比重によって通常、カイロミクロン（ＣＭ）、超低比重リポ蛋白（ＶＬＤＬ）、中間型リポ蛋白（ＩＤＬ）、低比重リポ蛋白（ＬＤＬ）、高比重リポ蛋白（ＨＤＬ）等の分画に分類される。そして種々の疾病や要因がこれらリポ蛋白の代謝に影響を与え、リポ蛋白分画の血中での増加や減少につながっている。このうちＬＤＬは血管壁へのコレステロールの蓄積に関与して動脈硬化作用を示すことが知られており、ＬＤＬ分画中のコレステロールは一般に悪玉コレステロールと呼ばれ、動脈硬化症や虚血性心疾患などの予防や診断のために測定される。

【０００３】従来ＬＤＬ分画の測定法としては、沈澱法、超遠心法、電気泳動法等が知られている。沈澱法はポリアニオンなどの沈澱剤を用いて分別沈澱させ、ＬＤＬ分画を測定するものであるが、超遠心法、電気泳動法と同様に操作が非常に繁雑であり、臨床検査の分野で汎用されている自動分析装置では利用できないという欠点がある。また１価のカチオンを有するポリビニルスルフェートを添加する方法（特開昭５８－４５５６２号）もあるが、やはり自動分析装置を利用することができない。

【０００４】特定分画に含まれる成分として臨床検査の分野では種々のものが確認されているが、とりわけ、コレステロール、トリグリセライド、リン脂質などがよく

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知られている。このうちで例えばコレステロールを定量する場合のコレステロール定量には、近年緩和な条件で使用でき、また反応の特異性が高い酵素法と呼ばれている酵素反応を用いる方法がもっぱら使用されている。これは、コレステロールエステラーゼ（ＣＥ）とコレステロールオキシダーゼ（ＣＯ）にパーオキシダーゼ（ＰＯＤ）と色原体を組合せて可視領域での吸光度を測定する方法と、ＣＥとコレステロール脱水素酵素（ＣＨＤ）に補酵素を組合せて紫外領域での吸光度を測定する方法に大別できる。

【０００５】

【発明が解決しようとする課題】前述のように従来のＬＤＬ分画の測定法では、自動分析装置への応用ができない。沈澱法は遠心分離による試料の分取工程が必要なためマルチチャンネルによる自動測定ができないことに加え、分取時に誤ってコンタミネーションを起す危険性も含んでいる。本発明の目的は、上述の問題点を改良して生体試料中のリポ蛋白の特定分画に含まれる成分を自動分析装置により定量することができる方法を提供することにあり、とりわけ、臨床検査におけるＬＤＬ分画中のコレステロールの有用な定量方法を提供することにある。

【０００６】

【課題を解決するための手段】本発明者らは鋭意研究した結果、特定のリポ蛋白分画を凝集させ、特定のリポ蛋白以外のリポ蛋白分画に含まれ当該定量すべき特定のリポ蛋白分画に含まれる成分と同じ成分を定量反応に関与しない別の反応系に導けば、その間特定のリポ蛋白自体は凝集しているため、この別の反応には作用しないことを見い出した。そして更に研究を重ねた結果、この後特定のリポ蛋白分画を少なくとも定量反応できる程度に解凝集して、当該成分を定量反応に付し、更に場合によっては定量反応を測定できる程度に特定のリポ蛋白分画を溶解して定量反応を停止できることがわかった。そしてこの定量反応により生じた変化、すなわち該成分又は該試薬の濃度変化、反応により生じた生成物、たとえば吸光又は発光物質の濃度などを測定することにより、前述の問題が解決でき、本発明の目的が達成できることがわかり、本発明を完成するに至った。

【０００７】即ち本発明は、生体試料中の特定のリポ蛋白分画に含まれる成分を直接定量する方法であって、特定のリポ蛋白分画を凝集させ、特定のリポ蛋白分画以外のリポ蛋白分画に含まれ当該定量すべき特定のリポ蛋白分画に含まれる成分と同じ成分を定量反応に関与しない別の反応系に導き、特定のリポ蛋白分画を少なくとも定量反応できる程度に解凝集して、当該成分を定量反応に付し、定量反応により生じた変化を測定することを特徴とするリポ蛋白分画に含まれる成分の定量方法を提供するものである。

【０００８】本発明で特定のリポ蛋白分画を凝集させる

には、凝集剤と抗体を用いるのが好適である。ここで凝集剤とは、化学反応によって凝集を惹起させるものであり、また抗体とは、特定のリポ蛋白分画に対する抗体であって、免疫凝集反応を生じさせるものである。このような凝集剤と抗体は、凝集させる分画によって単独で用いたり又はどちらかを先に加えた後で他を加えたり、同時に加えたりして組合せて用いることができる。このような凝集剤としては、本発明の目的が達成できるものであれば良く、例えば前述の沈澱法に用いられる沈澱剤のほか種々のものが利用できる。例えば血液中のリポ蛋白のうちＬＤＬ分画を凝集させる目的では、ポリエチレングリコール（ＰＥＧ）のほか、リンタングステン酸，デキストラン硫酸，ヘパリン等を単独で、又はMg^{++}，Mn^{++}，Ca^{++}，Li^{++}，Ni^{++}等のカチオンと組合せて用いることができる。

【０００９】また、抗体としては、本発明の目的が達成できるものであれば良く、例えばリポ蛋白のＬＤＬ分画を免疫凝集させる目的では、ＬＤＬ分画に対する抗体として、ＬＤＬ，アポＢ，β－リポ蛋白に対するポリクローナル抗体又はモノクローナル抗体の１種又は複数種を用いることができる。

【００１０】本発明で特定のリポ蛋白分画に含まれる成分を検出し定量する目的で用いる試薬には、臨床検査などの分野で公知の試薬が利用できる。例えばコレステロールを定量する目的では、前述の酵素法におけるＣＥ，ＣＯ，ＰＯＤを用いた試薬が好ましい。

【００１１】このような試薬を試料と定量反応させて当該反応を停止させるまでの反応時間は用いる試薬により異なる。反応を途中で停止させても終点で測定したものと定量値としては同じ結果が得られるものもあるが、通常１～６０分間から選択でき、例えばコレステロールを定量するためにＣＥ，ＣＯ，ＰＯＤを用いた場合は一般に２～３０分間から選択できる。

【００１２】本発明で当該定量反応を停止させるには公知の方法が利用できるが、例えば酵素法による場合はその酵素を阻害させるための阻害剤を用いることもできる。このような阻害剤としては、重金属，防腐剤，蛋白変性剤，界面活性剤等から選択できる。ＣＥ，ＣＯ，ＰＯＤを用いたコレステロールの定量では、アジ化ナトリウム，グアニジン塩酸等を例としてあげることができる。

【００１３】定量反応を停止する代わりに、定量反応が飽和した後に成分を検出してもよく、あるいは、反応の開始から一定時間後に成分を検出し、予め作成された検量線を用いて成分の量を測定してもよい。

【００１４】本発明で特定のリポ蛋白分画に含まれる成分と同じ成分を定量反応に関与しない別の反応系に導くための反応系としては種々のものをあげることができる。例えば前述のコレステロールを定量する目的でＣＥ，ＣＯ，ＰＯＤを用いた場合，これらの必要な酵素は

含むが発色に関与する試薬（色原体）の一部を除いたものを試薬として反応させると、無色の複合体を形成する。この後、目的とする定量反応を行うためＣＥ，ＣＯ，ＰＯＤおよび色原体のすべてを反応させても、無色の複合体はこれには関与しない。

【００１５】この例としてリポ蛋白の特定分画を凝集させたあとで、ＣＥ，ＣＯ，ＰＯＤおよび色原体としてＨＤＡＯＳ（正式名：Ｎ－（２－ヒドロキシ－３－スルホプロピル）－３，５－ジメトキシアニリン）を作用させると、特定のリポ蛋白分画以外のリポ蛋白分画に含まれる該成分であるコレステロールエステルとコレステロールはＣＥ，ＣＯの作用により過酸化水素を発生し、これにＰＯＤとＨＤＡＯＳが反応して無色の複合体を形成する。このとき、特定のリポ蛋白分画自体は凝集しているため、この反応には関与しない。次に特定のリポ蛋白分画をＣＥとＣＯが作用できる程度に解凝集し、ＣＥ，ＣＯ，ＰＯＤおよびＨＤＡＯＳと４－アミノアンチピリンからなる定量用試薬を反応させると、特定のリポ蛋白分画に含まれる該成分であるコレステロールエステルとコレステロールはＣＥ，ＣＯの作用により過酸化水素を発生し、これにＰＯＤ，４－アミノアンチピリン、ＨＤＡＯＳが反応して５８０ｎｍ付近の波長に極大吸収を有する色素を生成する。このとき、この反応の吸光度を測定できる程度に凝集を溶解させ、吸光度を測定することにより、特定のリポ蛋白分画に含まれるコレステロールが定量できる。

【００１６】この他の例として、定量反応とは異なる反応で定量反応には関与しない反応系を用いることもできる。例えば、定量反応に前述のＣＥ，ＣＯ，ＰＯＤの酵素反応を用いるとき、特定のリポ蛋白分画以外のリポ蛋白分画の成分とはＣＥ，ＣＨＤ及び$ＮＡＤ^{+}$の反応系を作用させ、ＮＡＤＨを生成させる反応系に導く。このあと、ＣＨＤを阻害させてこの反応が進行しないようにし、特定のリポ蛋白分画をＣＥ，ＣＯが作用できる程度に解凝集し、ＣＥ，ＣＯ，ＰＯＤおよびＨＤＡＯＳと４－アミノアンチピリンからなる定量用試薬により反応させ、前述のようにこの反応の吸光度を測定できる程度に凝集を溶解させ、吸光度を測定することにより、特定のリポ蛋白分画に含まれるコレステロールが定量できる。

【００１７】本発明で凝集した特定のリポ蛋白分画を少なくとも定量できる程度に解凝集させるには、溶解剤として界面活性剤，無機塩類などを単独又は複数種用いることができる。溶解剤の例として、トリトンＸ，アデカトール，塩化ナトリウム，塩化カリウム等を挙げることができる。例えばＬＤＬ分画を凝集させたあと、少なくとも定量できる程度に解凝集させるには、トリトンＸ－１００を用いることができる。また、場合によっては定量反応を測定できる程度に溶解させるには、溶解剤として蛋白変性剤，界面活性剤，無機塩類などを単独又は複数種用いることができる。その例として、グアニジンお

よびその塩を挙げることができる。

【００１８】

【発明の作用および効果】本発明の方法では、特定のリポ蛋白分画を凝集させ、定量を目的としないリポ蛋白分画に含まれる成分は定量反応に関与しない反応系に導き、特定のリポ蛋白分画を少なくとも定量反応できる程度に解凝集して定量反応させ、更に場合によっては定量反応を測定できる程度に溶解して定量反応を停止させ、この定量反応により生じた変化を測定するという操作を連続的に行うことにより、従来困難であった臨床検査での自動分析装置を用いたマルチチャンネル化による測定が可能となる。また定量用の試薬には公知の酵素を用いることができるため、反応時間を短縮でき、多検体処理も可能である。

【００１９】更に従来の沈澱法では遠心分離の上清を検体とするため余分な量の試料が必要で、試料である血清等も多く必要であるが、本発明の方法では試料を直接検体とするため検体量の微量化が可能で、多項目同時測定に適している。加えて、本発明の方法では光学的な測定が可能なため、臨床検査の分野で汎用されている多くの分析装置へ応用ができる等、本発明の方法は臨床検査に極めて有用な効果を奏するものである。

【００２０】

【実施例】以下、本発明をより詳細に説明するために実施例をあげるが、本発明はこれらに限定されるものではない。

実施例１

本発明の方法で、ＬＤＬ分画中のコレステロールを定量する例を示す。まず次の試薬を準備した。

試薬１（Ｒ１）：抗ヒトアポＢヤギ血清

試薬２（Ｒ２）：２０％ＰＥＧ４０００，１３単位／ml ＣＥ，１３単位／mlＣＯ，１.０単位／ml ＰＯＤ，０.２mg／ml Ｎ－（２－ヒドロキシ－３－スルホプロピル）－３,５－ジメトキシアニリン，０.０５％トリトンＸ－１００

試薬３（Ｒ３）：１％トリトンＸ－１００，０.１１mg／ml ４－アミノアンチピリン色原体

試薬４（Ｒ４）：７Ｍ－グアニジン塩酸，１Ｍ－グアニジンチオシアネート塩

【００２１】上述の試薬を用いて、ＬＤＬ分画中のコレステロールが１５８.４mg／dlの高値ヒト血清（Ｓ）を希釈して系列を作り、それを検体にして日立７０７０形自動分析装置で図１のパターンにより測定し、各希釈系列をグラフにした。その結果を図２に示す。この結果から、本発明の方法によればＬＤＬ分画中のコレステロールを直線性よく定量できることがわかる。

【００２２】実施例２

本発明の方法で、ＬＤＬ分画中のコレステロールを定量し、従来の方法での定量結果と比較した。本発明の方法に従い、実施例１の試薬を用いて、同様に新鮮なヒト血清（Ｓ）を検体にして日立７０７０形自動分析装置で図１のパターンにより測定し、標準液の測定値からＬＤＬ分画中のコレステロールの定量法［クリニカル・ケミストリー（Clin. Chem.）１８，４９９，１９７２］により、これらの血清中のＬＤＬ分画中のコレステロールを定量した結果と比較した。その結果を表１に示す。

【００２３】この結果から、本発明の方法によれば、従来のフリーデワルド法と測定値がよく相関しており（相関係数 ｒ＝０.９８７）、ＬＤＬ分画中のコレステロールが自動分析装置で定量できることがわかった。

【００２４】

【表１】

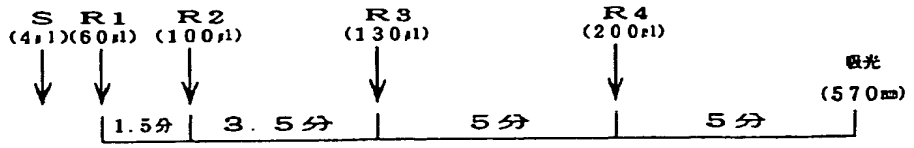
血清（No.）	本発明の方法 (mg/dℓ)	公知の方法 (mg/dℓ)
1	108.3	121.9
2	163.5	181.4
3	78.3	85.5
4	91.5	96.8
5	145.1	160.1
6	131.5	128.4
7	49.8	59.0
8	136.7	150.2
9	150.3	162.4
10	129.5	137.0
11	92.0	92.9
12	129.4	124.4
13	119.5	122.4
14	153.1	164.2
15	195.0	207.2
16	116.1	123.5
17	116.7	134.3
18	186.4	203.3
19	183.5	190.9

【図面の簡単な説明】

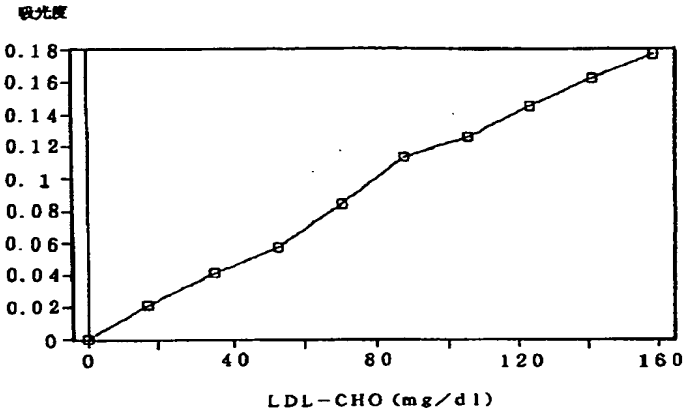
【図１】　自動分析装置における検体および試薬投入パターンを示す図。

【図２】　実施例２の結果を示すグラフ。

【図１】


S
(4μl)
R1
(60μl)
R2
(100μl)
R3
(130μl)
R4
(200μl)
吸光
(570nm)
1.5分
3.5分
5分
5分

【図２】


吸光度
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
40
80
120
160
LDL-CHO (mg/dl)